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Industrial Crops and Products 22 (2005) 15–25

INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL

www.elsevier.com/locate/indcrop

Breeding guayule for commercial production

Dennis T. Ray[a,*], Terry A. Coffelt[b], David A. Dierig[b]

[a] *Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA*
[b] *USDA, ARS, U.S. Water Conservation Laboratory, 4331 E. Broadway Road, Phoenix, AZ 85040, USA*

Received 14 January 2004; accepted 26 June 2004

Abstract

Breeding a new domestic crop, such as guayule, is not appreciably different from enhancement and breeding of conventional crops. In both instances, plant breeders take the available germplasm and search for genetic variability in the desired traits. The major differences are that in new crops plant breeders are often working with an unfamiliar species that is not yet fully domesticated and the available germplasm is often limited.

The main objective of the guayule breeding program is to facilitate successful commercialization by developing higher yielding cultivars. Improvement has been accomplished, with newer lines yielding up to 250% more rubber than lines developed in the 1940s and 1950s. This is surprising because the genetic base from which improvements have been made appears to be very narrow, and because guayule reproduces predominately by apomixis (asexual reproduction by seed). Improvements through breeding are dependent upon genetic diversity within the available germplasm, and being able to identify different genotypes. The available germplasm exhibits extreme variability both within and between lines for morphological traits such as height, width, and biomass; chemical constituents such as rubber, resin, and latex contents; and genetic and chromosomal markers. The measured variation is due partly to the facultative (asexual reproduction and sexuality coexisting) nature of apomixis in guayule, which periodically releases genetic variation among progeny. A great amount of this measured variation is due to environment, and selections, to take advantage of genetic differences, must be made within the first 2 years of growth. There have been relatively few individuals involved in guayule breeding. Thus, with limited resources and time, most of the improvements have been made through single-plant selections from within populations. Although this method has the potential for only modest long-term gains, improvements occur relatively quickly. Indirect measures have been developed to increase breeding efficiency. For instance, most selections are made for plant height, width and biomass because they are highly correlated with rubber yield. As guayule approaches commercialization, breeding will become a priority and other breeding schemes will be tested and utilized such as: mass selection; recurrent selection among sexually reproducing diploids, followed by chromosome doubling; family selection; crossing high yielding apomictic plants; and crossing high yielding apomictic plants to sexual diploid plants to release new genetic combinations.

Keywords: Guayule; *Parthenium argentatum*; Plant breeding; Apomixis; Genetic diversity; Yield components; Germplasm; Rubber; Resin; Latex

* Corresponding author. Tel.: +1 520 621 7612; fax: +1 520 621 7186.
E-mail address: dtray@u.arizona.edu (D.T. Ray).

0926-6690/$ – see front matter.
doi:10.1016/j.indcrop.2004.06.005

1. Introduction

Guayule (*Parthenium argentatum* Gray) is a potential economic and renewable source of rubber/latex. This paper updates two previous reviews (Thompson and Ray, 1988; Estiali and Ray, 1991) on the genetics, germplasm development, and breeding of this new crop. The primary objective of the guayule breeding programs has been to facilitate successful commercialization by developing higher yielding cultivars. However, other approaches and information are necessary for full commercialization, and much of this research has been led by Dr. Francis Nakayama, who we honor with this symposium. Dr. Nakayama has contributed significantly to guayule's development, especially in the areas of agronomy (planting, irrigation and harvesting), development of chemical procedures to extract rubber/latex (for both laboratory analyses and for commercial production), and most recently in his research on the pesticidal properties of the bagasse and byproducts left after latex extraction. For plant breeding programs, Dr. Nakayama's main contribution has been the development of chemical testing procedures to evaluate new lines.

Guayule is one of over 2000 rubber producing species, however only two, *Hevea brasiliensis* (A. Juss.) Muell.-Arg. and guayule have been exploited as commercial sources of natural rubber. Today, *Hevea* is an established and greatly improved crop, acclimated to growth in areas outside of its natural habitat, and essentially the sole source of natural rubber for industry. In contrast, work is still underway to completely domesticate and commercialize guayule as a new or alternative crop for arid and semiarid areas of the southwestern United States, north central Mexico, and regions with similar climates throughout the world (Thompson and Ray, 1988).

Although *Hevea* is the dominant rubber crop today, *Hevea* and guayule had parallel early histories of development. In both, commercialization began by harvesting from wild stands, before the establishment of plantations and the initiation of cultural studies. Variability within wild stands lowered yields in both species, and this problem continued through the early attempts at cultivation because populations were established with open-pollinated seeds collected from plants that were very heterogeneous genetically. The differences in commercial development between the two crops can be associated with the initiation of the Rubber Research Institute of Malaya in 1925, which has been responsible for over 75 years of continuous increases in *Hevea* yields and the production of a uniform and reliable industrial product (Bonner, 1991). Guayule, on the other hand, has suffered from intermittent research efforts, which have been undermined by periods of neglect. Guayule researchers have found themselves more than once in the position of "reinventing the wheel," whereas *Hevea* has had a continuous and well supported research effort (Ray, 1993).

2. History of guayule domestication and commercialization

Guayule has been known as a source of rubber since pre-Colombian times when native populations in Mexico used it to form balls for their games. In the early 1900s, guayule was considered as an alternative source of natural rubber in the United States because of the high price of *Hevea* rubber from the Amazon region (Bonner, 1991). Since then, there have been four major efforts to domesticate and commercialize guayule. The first effort to commercialize guayule began at the turn of the 20th century with the harvesting of wild stands in Mexico. Several extraction methods were evaluated, and in 1902, 40 kg of guayule rubber were exported to the United States. By 1907, a total of 20 extraction plants were either operational or under construction in Mexico, and in 1910, Mexican rubber production reached a peak of 10000 t, which accounted for 24% of the total rubber imported into the United States (Bonner, 1991). Production by the Continental Rubber Company continued until 1912, when the Mexican Revolution halted all production. This may have been a blessing in disguise because very large areas of native shrubs had been depleted, and guayule was on its way to becoming extinct in the wild.

After the closure of the processing facilities in Mexico, commercialization efforts were moved into the United States. Agronomic and breeding studies were initiated by the newly reorganized Intercontinental Rubber Company, with efforts centered in Arizona and California. By the late 1920s, annual production of guayule rubber was about 1400 t from commercial plantings of over 3200 ha. Seventeen years (1929) after the project moved from Mexico to the United States, the first effort to commercialize guayule ceased due to the Great Depression (Bonner, 1991; Ray, 1993).

The second effort to domesticate and utilize guayule as a source of natural rubber came with the Emergency Rubber Project during World War II. This was an extensive effort involving over 1000 scientists and technicians and 9000 laborers, with over 13000 ha of shrub planted at 13 sites. The effort ended with the end of the war and the development of synthetic rubber (Huang, 1991). During the 4 years of the project's existence, 1400 t of guayule rubber were produced, but at the end of the war an additional 10000 t of rubber were destroyed while still in the shrub (Bonner, 1991). Scientifically, the project was very successful, and it provided the bulk of our knowledge about guayule's basic biology and the origin of the germplasm upon which the current breeding programs are based (Hammond and Polhamus, 1965; Thompson and Ray, 1988).

The third effort arose from the quadrupling of crude oil prices in the early 1970s. This led to the enactment of the Native Latex Commercialization and Economic Development Act of 1978, which supported guayule research for about 12 years (Huang, 1991). Although this effort was neither as concentrated nor as urgent as the Emergency Rubber Project, a tremendous amount of work was done, and resulted in significant increases in yield and refined cultural practices to fit today's mechanized agriculture (Whitworth and Whitehead, 1991). Thus, guayule could substitute for *Hevea* natural rubber in a variety of high temperature applications, especially tires, but no great push was made to commercialize guayule because the manufacturers of these products were for the most part the owners of the *Hevea* plantations, and supplies appeared plentiful.

The fourth and present effort to commercialize guayule came about as a result of the widespread occurrence of life-threatening "latex allergy" attributed to *Hevea* rubber products in the 1990s. To alleviate this problem, guayule was shown to be a source of hypoallergenic latex that could replace *Hevea* latex (Siler and Cornish, 1994; Cornish et al., 1996). In addition to the desire for hypoallergenic latex products, the demand for natural rubber is increasing worldwide, and the production from *Hevea* plantations will not be able to keep pace with demand. The political instability in the areas where *Hevea* rubber is grown and imported (Southeast Asia) is a concern to end users, plus the need for a reliable domestic source is again of major consideration. Furthermore, the development of additional products from guayule such as termite resistant wood products, resin-based products, and bio-energy uses make guayule an attractive new crop for production in the arid and semiarid areas of the world in addition to being a source of rubber (Bultman et al., 1991; Kuester, 1991; Nakayama et al., 2001, 2003).

3. Breeding

Guayule and *Hevea* have similar early breeding histories. Yields were first increased by planting larger areas and improving cultivation techniques rather than through breeding. Both are difficult species to work with from the breeder's point of view because they are perennials, and thus, need a considerable amount of land for breeding programs. Also, both species are physiologically immature for 3–7 years before the first harvest, and in both reproduction is essentially asexual (clones in *Hevea* and apomicts in guayule). They both have the potential for continuous production, tapping in *Hevea* and harvest of regrowth in guayule, which is a consideration in breeding programs because breeding plots must be maintained for many years. The first breeding successes in both were through mass selection, grafting of high yielding clones in *Hevea* and selection of high yielding plants in guayule. Finally, the available germplasm from which selections have been made in both species was very narrow (Tan, 1987; Thompson and Ray, 1988; Bricard and Nicolas, 1989; Henon and Nicolas, 1989; Annamma Varghese, 1992; Ray, 1993; Clement-Demange et al., 2001).

Plant breeding, which probably predates civilization, has been practiced as an art for at least the past 10,000 years, but as an academic discipline for only about a century. Today we use sophisticated hybridization and selection schemes to increase significantly yields and introduce new quality traits not found in early cultivars, such as disease and insect resistance, new levels or kinds of stress tolerance, as well as greater yield capability. Breeders continue to enhance gradually the available genetic materials (germplasm), slowly making them more amenable to cultivation and human use. Thus, we can say that in this regard breeding for improvement of new industrial crops is not appreciably different from that of the more established food, feed, and fiber crops (Thompson, 1990).

In both new and established crops, breeders take the available germplasm and search for genetic variability in desired traits. This is the way plants have been improved throughout history, as was pointed-out by Darwin (1868) over a century ago in his book *The Variation of Plants and Animals Under Domestication*.

"... although man did not cause variability and cannot prevent it, he can select, preserve, and accumulate the variations given him by the hand of nature almost any way he chooses; and certainly produce a great result."

"... domestic races of plants often exhibit an abnormal character, as compared with natural species; for they have been modified not for their own benefit, but for that of man."

Although Darwin did not understand the source of genetic diversity, he did understand that we can alter plant populations as needed by accumulating and using the variations found in nature.

In order to maximize the commercial potential of any crop, a plant must first be domesticated. Domestication is the unique process by which a plant species is adapted to a life of intimate association with and for the advantage of humans. In other words, characteristics that are advantageous to a plant in the wild may not be the best for optimum production, and must be changed through breeding. Some of the wild characteristics that most often need changing are: seed characteristics (shattering, small size, dormancy, irregular germination); flowering (indeterminate, day-length sensitivity); morphology (branching habit, vegetative/reproductive ratio, growth rate, biomass yield); limited growing range; and disease and insect susceptibility.

In both *Hevea* and guayule, there have been successes through plant breeding, shown by dramatic increases in latex yield from 400 to over 3000 kg/ha for *Hevea* (Bonner, 1991), and from 300 to 1000 kg/ha rubber for guayule (Estiali and Ray, 1991). However, *Hevea* has been changed significantly, and is considered a domesticated crop, whereas guayule still contains many wild characteristics that are deterrents to full commercialization.

4. Reproductive biology

Guayule is one of the dominant perennial xerophytic shrubs found on the limestone hillsides of the Chihuahuan Desert of northcentral Mexico and the Big Bend region of Texas (West et al., 1991). Wild stands contain a natural polyploid series of diploids ($2n = 36$), triploids ($3n = 54$) and tetraploids ($4n = 72$); and under cultivation individual plants have been identified with chromosome numbers up to octaploid ($8n = 144$) (Thompson and Ray, 1988). Diploids reproduce predominately sexually, and polyploids reproduce by facultative apomixis (apomixis and sexuality co-existing). Guayule has a sporophytic system of self-incompatibility and many plants contain B- or super-numerary chromosomes (Thompson and Ray, 1988).

In apomictic guayule, the embryo sac develops directly from the megaspore mother cell (MMC) without meiosis. Pollination is not necessary for embryo development, but is needed for normal endosperm and seed development (Powers, 1945). Meiosis in microspore mother cells is normal, reducing the chromosome number in male gametophytes. It has been assumed in guayule that apomixis would assure genetic uniformity from generation to generation. However, its facultative nature and the high amount of heterozygosity in individual plants and the heterogeneous make up of populations, releases considerable variation whenever sexual reproduction (amphimixis) occurs (Powers and Rollins, 1945).

The facultative nature of apomixis in guayule results in four classes of progeny (Esau, 1946; Ray et al., 1990). The origin and relative chromosome numbers of these four classes from tetraploid parents ($4n = 72$) illustrate the complexity of reproduction and the potential for release of genetic variability in this species. In class 1 (generally the predominant class), progeny arise from non-reduction of the MMC without fertilization. This results in apomictic tetraploid ($4n = 72$) progeny that are identical genetically to the maternal parent. Class 2 progeny, resulting from fertilized, unreduced MMCs, includes plants with increased ploidy levels (e.g., hexaploid progeny, $6n = 108$). In class 3 polyhaploids ($2n = 36$), plants are the result of meiotic reduction of the tetraploid MMC, and embryo development without fertilization. Finally, class 4 results in amphimictic (sexual) tetraploid ($4n = 72$) progeny that arise from normal meiotic reduction and fertilization (normal sexual reproduction). Thus, there are two reproductive modes that produce tetraploid progeny, one by apomixis and the other by sexual reproduction. This means that a plant breeder cannot differentiate between the apomictic and sexual progeny by chromosome number alone. The remaining two progeny classes vary in chromosome number from the parental population and are fairly easy to identify.

Even though Powers and Rollins (1945) reported that reproduction in guayule occurs by facultative apomixis, the relative amounts of sexuality and apomixis was unknown. Thus, it was surprising that for many growth characters a great deal of variability was found among progeny of single plants (Thompson et al., 1988). The source of this variation could be either environmentally or genetically influenced because apomixis is facultative.

Keys et al. (2002) attempted to develop a rapid method to evaluate apomictic potential (inversely sexuality) in guayule. Although the method is neither simple nor rapid, apomictic potential/sexuality can be estimated. Among seven lines investigated, three were found to be very apomictic, two with medium apomictic potential (a mix of apomixis and sexuality, but mainly apomictic), one with low apomictic potential (mainly sexual reproduction), and one essentially sexually reproducing. The facultative nature of apomixis in guayule allows for the periodic release of variability that can be exploited by breeders.

5. Germplasm

The domestication and development of guayule as a crop was initiated in 1910 by W.B. McCallum, who was then employed by the Intercontinental Rubber Company. A breeding and selection program was started in 1916 at Continental, Arizona, and transferred to Salinas, California in 1925. A selection made by McCallum in Salinas, '593', was the principal cultivar utilized in production in the 1920s, 1930s, and the Emergency Rubber Project during World War II (Thompson and Ray, 1988). During the Emergency Rubber Project one major activity was germplasm and cultivar development, and the breeding material developed during this time became the basis for the research efforts starting in the 1970s and continuing until today (Thompson and Ray, 1988).

The first collection of guayule germplasm was made by McCallum in 1912. Because of the civil strife and revolution in northern Mexico, wild plant material would not be available to guayule workers in the U.S., so that McCallum was instructed to gather seeds from wild stands and move his cultural operations to a location near San Diego, California. Although the Mexican government refused to allow McCallum to carry seeds from the country, he was able to bring seeds into the U.S. by hiding them in a tobacco tin, which the border guards ignored when he was searched at the border. The seeds were initially planted at Valley Center, California, and evaluation of germplasm was subsequently conducted at Continental, Arizona and Salinas, California (Thompson and Ray, 1988).

There were two main germplasm collection expeditions during the Emergency Rubber Project. Powers, McCallum, and Olson collected 66 accessions from 24 locations in Mexico; and Powers and Federer collected 368 accessions from 21 locations in Texas. These accessions were then planted and evaluated at Salinas in 1943. In 1948, Hammond and Hinton collected an additional 174 accessions from 93 locations in Mexico (Thompson and Ray, 1988).

The USDA guayule breeding program at Salinas, California was terminated in 1959, and 24 germplasm lines, developed by H.M. Tysdal from the Powers, Hammond and Hinton collections, plus line '593' developed by McCallum, were selected for storage at the USDA National Seed Storage Laboratory (now the National Center for Genetic Resources Preservation) at Fort Collins, Colorado in 1965. These 25 lines, selected on the basis of their rubber production and plant-growth characteristics, were the only ones saved from the hundreds of selections, breeding lines, and accessions stored at Shafter and Salinas, California. These 25 lines plus the line 'Bulk Richardson' (from D.D. Rubis; a bulk seed collection from Mexico made by Richardson) became what was commonly called the 26 USDA germplasm lines, from which the breeding programs in the 1970s began (Thompson and Ray, 1988).

It is of considerable interest to note that 21 of the 26 USDA lines came from the state of Durango, Mexico. The apparent narrow germplasm base is accentuated by the fact that 15 of the lines descended from the Powers, McCallum, and Olson collection #4265, which was a bulked seed collection from five plants at one location. The original diploid material came from collection

#4254, which was also bulked seed from five plants at one location (Thompson and Ray, 1988).

In 1976, R.C. Rollins made collections from 45 locations in Mexico. In 1977, C.T. Mason collected related *Parthenium* species throughout Mexico and Naqvi and Hanson collected guayule from 50 locations in Mexico, and in 1982 Tipton and Gregg collected seeds from 10 native populations in Texas. An extensive effort was mounted in 1982 by Mexican scientists who collected 3000 accessions from 310 locations from six states (Thompson and Ray, 1988). Unfortunately, it is unclear where most of these accessions are today, and many of those that we do have access no longer have viable seeds.

All breeding approaches depend upon the existing genetic variability found in the available germplasm. However, in guayule, this genetic base appears to be very narrow, but this has not been a hindrance to guayule breeding programs because the facultative nature of apomixis in polyploid guayule continually releases new variability with each seed harvest. In fact, with the limited scale of the present plant improvement programs, this variability is created faster than it can be exploited by breeders (Thompson and Ray, 1988).

Most guayule germplasm today consists of apomictically reproducing triploid ($3n = 54$) and tetraploid ($4n = 72$) accessions, which have received most of the attention in breeding programs (Hammond and Polhamus, 1965; Thompson and Ray, 1988). Sexually reproducing, largely self-incompatible diploids ($2n = 36$), have had only limited use in guayule breeding programs.

At present, the USDA-ARS, National Arid Land Plant Genetic Resources Unit in Parlier, California has 144 *P. argentatum* accessions and five interspecific hybrids of different *Parthenium* species. Twenty-five of these accessions have PI numbers, with the rest carrying western regional numbers, and as many as 64 accessions may not have viable seed. This is an important problem that needs to be addressed by the National Plant Germplasm System.

6. Genetic diversity in guayule germplasm

Plant materials derived from the preceding germplasm collections have exhibited extreme variability both within and between lines for rubber quality and quantity, dry weight, resin content, latex content, and yield (Naqvi, 1985; Thompson and Ray, 1988; Thompson et al., 1988; Dierig et al., 1989a, 1989b; Coffelt et al., 2004), chromosome number (Powers, 1945; Bergner, 1946; Thompson and Ray, 1988; Cho, 1993) and isozymes (Estilai et al., 1990; Diallo, 1993; Ray et al., 1993). However, much of the measured variability for rubber and resin production, and growth appears to be due to environmental influences (Dierig et al., 2001; Coffelt et al., 2004). Chromosome number and isozymes are considered environmentally neutral, and thus, any differences found for these characteristics must be a measure of genetic differences among plants.

6.1. Chromosome numbers

Polyploidy and aneuploidy are common in guayule (Powers, 1945; Bergner, 1946; Thompson and Ray, 1988). Cho (1993) observed the number of chromosomes among 15 progeny in 12 different families, each derived from a single-plant selection. He found that when all families were combined 77.3% of the progeny were tetraploid, 4.5% were polyhaploid and the rest had different aneuploid chromosome numbers up to 81. From these data, he estimated meiotic reduction at 9% and fertilization at about 22%. Among all families the frequency of tetraploid progeny ranged from 47% to 100%.

6.2. Isozymes

Isozymes are well described genetically and are not influenced by the environment, thus differences among progeny are a measure of genetic variation. Variation in either banding patterns or intensity of bands (polymorphisms) allows for the estimation of the amount of out-crossing and meiotic reduction that occurs in guayule.

Estilai et al. (1990) used six isozyme markers to identify genetic differences among diploid guayule entries. They found variation within entries, indicating that most of the available diploid guayule germplasm and selections were heterogeneous, and they suggested that isozymes may provide useful markers for guayule cultivar identification.

Diallo (1993) found that there was variation for isozyme banding patterns among progeny of single apomictic plants for the isozymes esterase and peroxidase. Six progeny from 20 different single-plant selections were evaluated. Sixteen of the 20 six-plant families were polymorphic for esterase, and 18 of the 20 polymorphic for peroxidase. Overall, 37% of all progeny showed polymorphisms for esterase and 13% for peroxidase.

7. Components of yield

Selection in guayule has been significantly aided by the description of the components of yield and their relationships to rubber production (Thompson et al., 1988; Dierig et al., 1989b). In general, rubber content (%) was not found to be highly correlated with rubber yield, and in fact was often negatively correlated. Fresh and dry weights, as well as other characters related to biomass production, were highly and consistently correlated to rubber yield (Thompson et al., 1988; Dierig et al., 1989b). The characters shown to be the best predictors of rubber content were plant fresh and dry weight, percent dry weight and plant volume, and the best predictive model for rubber yield includes plant height and width, volume and dry weight (Dierig et al., 1989b).

Ray et al. (1993) tested the relatedness of apomictic parents and their open-pollinated, half-sib progeny families for eight components of yield. Heritability estimates were made by measuring the components of yield in both the parents and progeny. The parent plants were all open-pollinated progeny of a single-plant selection made by D.D. Rubis, and measurements were made when the parent plants were 3-year-old, and the progeny plants 2-year-old. For rubber yield, rubber content, resin content, fresh weight, dry weight, percent dry weight, height and width, none of the parent-progeny regressions were significantly different from zero. For all characters, a large range of phenotypic variation was observed, and the range and standard deviation of the parents were greater than among the progeny. This was probably due to the compounding of environmental effects (the parent plants were a year older than the progeny plants), rather than a difference in genetic variability (Dierig et al., 2001). Linear correlations were performed to study the relationship between rubber yield and the other seven characters, and fresh and dry weights were highly and positively correlated with rubber yield in all populations. Thompson et al. (1988) found significant correlations between rubber content and resin content that were higher than correlations of any other character with rubber content. This high correlation means that breeders should be able to create new lines that are higher in both rubber and resin than older lines. Because both rubber and resin are important characters in determining the value of guayule end products, breeding for simultaneous increases in these traits is important to insure commercialization. Evidence that this is possible is found in the release of six new germplasm lines that are higher in rubber and resin the older USDA lines (Ray et al., 1999).

Biomass appears to be the best predictor of rubber yield (rubber yield = plant biomass × rubber concentration). Thus, plant growth or biomass production can be used as a primary selection index for rubber yield. However, selection for large plant size may be disadvantageous, because harvesting the shrub in the field, its transportation and handling, and the efficiency of rubber extraction in the processing plant are significant economic factors in the production of rubber from guayule. For this reason, selection of plants with higher rubber concentration in concert with adequate biomass production must receive primary attention. Such selection is difficult because there is often a negative correlation between rubber concentration and biomass (Thompson et al., 1988).

8. Potential breeding approaches

In many instances, the breeding of new and conventional crops is the same. The major differences are that in new crops: (1) the plant breeder starts with a different, and frequently unique and exotic germplasm base from which to develop a crop; (2) the breeder is often totally unfamiliar with the species, the germplasm, and potential end products; (3) the traits to be improved frequently have not been identified by researchers, industry, or growers; and (4) there is often a paucity of previous research, including the appropriate technology for evaluating, selecting, and breeding of the products and coproducts sought. New crop breeders must be flexible in their approach to breeding where so much is unknown. The breeder must be innovative and able to change approaches and methodology rapidly to meet the opportunities and constraints as they are encountered.

The primary objective for all guayule breeding programs has been to increase rubber yield, but recently this has shifted to increase the latex portion because this fraction will be used to produce hypoallergenic products. Secondary objectives have included improving rubber quality, seedling and mature plant vigor, plant architecture, regeneration following harvest by clipping, and tolerance to salinity, drought, diseases, and pests (Thompson and Ray, 1988; Estiali and Ray, 1991). However, because of the relatively few researchers involved in guayule breeding, the secondary objectives have not received much attention over time.

The most extensively employed breeding approach in guayule has been single-plant selections from within apomictic polyploid populations. Selection of individual plants is usually the simplest and most rapid method when heritabilities for desired characters are high. If heritabilites are high, increases can be made in a short period of time, but the long term potential is for only modest gains because apomixis restricts the release of new genetic combinations. This has been the primary method employed because breeders were under considerable pressure to increase rapidly rubber content and yield, and there were relatively few individuals doing the work. Thus, the degree of success using this method depends first upon the amount of heterogeneity in the population; second, whether or not the differences are genetic; and third, on the number of plants that can be screened (Thompson and Ray, 1988). This method increased annual rubber yields from approximately 300 to 1000 kg/ha, by selecting for the components of yield described previously, but predominately by selecting simultaneously for rubber concentration (%) and dry matter or biomass production (Estiali and Ray, 1991).

When heritabilities are low, single-plant selection is not as effective as family selection. In family selection, families of progeny, either full-sibs or half-sibs, are used to evaluate the quality of the parent plants. Thus, parent plants are not selected on their own merits, but on those of their progeny. The disadvantage of family selection is that there is a lengthened generation interval. However, because guayule is a perennial plant with almost continuous flowering, many generations of progeny can be obtained from a single plant once it has been selected as a suitable parent.

Breeding guayule must go beyond individual plant selections. Hybridization of apomictic polyploids is a method that has been suggested, but has been used sparingly because of the problems of separating the offspring that arise from sexual reproduction from the apomicts. Plants expressing high levels of sexuality could be identified using the method of Keys et al. (2002), and crossed to produce new genetic combinations from which further selections could be made. Seed would be collected from the hybrid plants, planted, and tested for apomictic potential. If the resulting progeny are predominately apomictic, seed from them would be placed in progeny trials and tested for possible release as new lines. If the plants are predominately sexual, they could be backcrossed to enhance certain characteristics, self-pollinated to produce a segregating population from which more selections could be made, or apply standard breeding strategies generally not used in guayule.

Interspecific hybridization has been applied on only a limited scale. Until more resources and personnel are available for guayule breeding, there appears little to be gained by going to related species to find desirable traits because the available variability within the guayule germplasm has not yet been totally characterized and/or exploited (Estiali and Ray, 1991). Also, none of the other *Parthenium* species produce an appreciable amount of rubber, although they should be considered as potential sources of vigor, increased resin content, increased biomass, disease and insect resistance, regrowth ability after clipping, and cold tolerance. The major disadvantage would be that it would take a large number of backcross generations of the interspecific hybrid to guayule to increase the rubber content as well as to keep the new desirable trait(s).

The University of California-Riverside has released three germplasm lines (Cal-1, Cal-2, and Cal-5) that were developed from interspecific crosses of guayule with three different *Parthenium* species. These three have increased vigor, biomass production, and resistance to verticillium wilt. AZ-101, a vigorous interspecific hybrid, is an open-pollinated cross between guayule and *Parthenium tomentosum* var. *stramonium* (Thompson and Ray, 1988; Estiali and Ray, 1991).

Mass selection is one of the oldest plant breeding methods, and certainly has been used in various forms for 10,000 years. With mass selection, significant gains can be achieved in a relatively short period of time because only the top yielding plants in a population are selected to become the parents of the next generation. Today, mass selection is used to enhance germplasm and develop cultivars, especially in crops where there are few individuals involved and cross pollination is the major mode of reproduction. Mass selection has been used in sexual diploid populations by Ray et al. (1995), in which, after three cycles of selection, a diploid line was developed that is tolerant to *Verticllium dahliae*.

Mass selection has never been used extensively in polyploid guayule because, to enhance populations using this method, one must be able to screen effectively many plants (hundreds at minimum, to thousands optimally), the resulting progeny must be uniform, and cross pollination must be the major mode of reproduction. Many of the important characters in guayule appear to be multigenic, in which heritability is low, and screening procedures for large numbers of samples have not been developed. In addition, polyploid guayule populations are fairly variable because of new genetic combinations resulting from facultative apomixis in highly heterozygous plants, and from environmental effects compounded over several years of growth. The apomictic nature of polyploid guayule greatly reduces the chance of attaining significant gains using mass selection.

Diploids have been used in guayule breeding because of their sexual (non-apomictic) reproduction, and thus the ability to use standard breeding methodologies. While there are problems in using diploids, such as significantly lower yields and increased susceptibility to root diseases, these yield and disease problems have been overcome by using modified recurrent selection schemes to increase yield, and mass selection to develop *Verticillium* tolerant lines (Estiali and Ray, 1991; Ray et al., 1995). These improved diploid lines can either be crossed to apomictic polyploids or have their chromosome numbers doubled with colchicine. Diploids could also be used to release new genetic combinations by crossing them as the female parent to apomictic polyploids. The resulting apomictic progeny plants might contain new and useful combinations of genes, because meiosis in the microspore mother cells of the apomictic polyploid male plants is normal. Once high yielding polyploids are identified, they could be crossed onto diploids resulting in populations with enough variation from which to make selections (Thompson and Ray, 1988; Estiali and Ray, 1991).

A potential breeding method that can make the most of limited resources in guayule is the pedigreed natural crossing method (Hammons, 1964; Coffelt, 1989). Guayule meets the requirements for use of this method by having natural cross pollination between potential parents (species or diploids or polyploids) and dominant markers to identify hybrids. The advantages of this method are that crossing is not dependent on limited time available for a single scientist or trained assistant to perform the cross; identification, harvesting, and isolation of hybrids can be done by semi-skilled workers on land unsuitable for yield trials and other experiments; and it is more economical than making crosses in the greenhouse. The biggest disadvantages are that the pedigree of the hybrids is based on a parental line rather than a single plant and large amounts of land may be needed to identify hybrids. The advantages of this method of producing large numbers of hybrids with little effort should outweigh the disadvantage of individual parent plant identification. The higher outcrossing rate of guayule compared with self pollinated species should result in a larger number of hybrids being identified with the same amount of land.

Yield trials have been used successfully to evaluate guayule germplasm lines under various environmental conditions (Estiali and Ray, 1991). However, this valuable tool was lost to breeders as funding was eliminated for some projects. Recently, new yield trials have been established as a result of an Initiative for Future Agriculture and Food Systems consortium grant to the University of Arizona (Majeau et al., 2003). More consistent funding is needed to carry these trials to completion and initiate new ones as new germplasm becomes available.

Progress in selection for rubber/latex traits has been hampered because of the difficulty in determining rubber and latex yield in single plants. The analyses for rubber and latex contents is a labor intensive and time consuming process, greatly limiting the number of samples that can be processed. The amount of leaves, the moisture content of the shrub, and deterioration of the latex during processing, all can interfere, and must be considered, in the analysis of rubber and especially latex (Teetor et al., 2003). In addition, morphological traits have not been identified that consistently correlate with rubber or latex content. Improvements in these areas could greatly speed the breeding progress.

Research is needed to establish the relationship between latex and rubber concentrations and yields. If rubber and latex concentrations and/or yields are closely related, then previous relationships established between rubber concentration/yield and the various yield components can be expected to be the same as their relationships with latex concentration/yield. However, if rubber concentration is not closely related to latex concentration, then studies will need to be conducted to establish the relationships between latex concentration and traits such as plant biomass, latex yield, rubber concentration and yield, resin concentration and yield, plant height and width, etc. Recent studies (Coffelt et al., 2003) showing that latex concentration and yield vary with storage conditions prior to chipping, whereas rubber concentrations and yield do not, suggest that research defining the relationships between latex concentration and yield will need to be done before meaningful breeding programs can be started.

9. Conclusion

Full commercial production and utilization of guayule will be greatly facilitated by the development of higher-yielding cultivars that fit well in today's mechanized agriculture. In spite of limited personnel and funding resources, yields have been improved significantly. There is abundant genetic variability from which further progress may be made. However, as guayule becomes a commercial crop, the demands upon the plant breeders will be greater. Generally, the more successful a crop, the greater the demands on, and at times complaints about, breeders (Duvick, 1990).

In the past guayule has suffered from intermittent support, and advancements often undermined by periods of indifference. If guayule is to become a commercial crop, it is important that a sustained level of support for plant breeding is maintained. As in almost all commercial crops today, funding for guayule plant breeding will have to come from the developing industry because public funds for this type of research are minimal.

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